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<b>(54) Title:</b> VECTOR CONSISTING OF A TRANSCRIPTIONAL REGULATORY DNA SEQUENCE LINKED TO A DNA SEQUENCE ENCODING BETA-LACTAMASE FOR ENZYME PRODRUG THERAPY		
<b>(57) Abstract</b>  The invention relates to a molecular chimaera for use in therapy with a prodrug, the chimaera comprising a transcriptional regulatory DNA sequence capable of being activated in a targetted mammalian cell and a DNA coding sequence operatively linked to the transcriptional regulatory DNA sequence and encoding a $\beta$ -lactamase enzyme such that on expression of said coding sequence in the targetted cell, the $\beta$ -lactamase enzyme is capable of catalysing conversion of the prodrug into an agent toxic to the targetted cell. The enzyme $\beta$ -lactamase has particular advantages when used in GDEPT or VDEPT in terms of the range of prodrugs that can be produced which are capable of being converted to the active species by the enzyme.		

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VECTOR CONSISTING OF A TRANSCRIPTIONAL REGULATORY DNA SEQUENCE LINKED TO  
A DNA SEQUENCE ENCODING BETA-LACTAMASE FOR ENZYME PRODRUG THERAPY

The present invention relates to targetted enzyme prodrug therapy.

5 Targetted enzyme prodrug therapies provide a method for restricting the activity of a  
chemotherapeutic agent to a particular target site. This is desirable when the systemic  
presence of the chemotherapeutic agent produce unwanted side effects. Although  
applicable to any therapeutic regime for which a targetted approach is advisable, the  
technique is particularly applicable to the treatment of cancer where therapeutic  
10 regimes have previously involved the systemic introduction of highly cytotoxic  
compounds which exert their effect in a non-selective manner on both healthy and  
tumourogenic cells.

Research in the area of cancer chemotherapy has produced a variety of antitumour  
agents which have differing degrees of efficacy. Standard clinically useful agents  
15 include adriamycin, actinomycin D, methotrexate, 5-fluorouracil, cis-platinum,  
vincristine and vinblastine. However, these presently available antitumour agents are  
known to have various disadvantages such as toxicity to healthy cells and resistance  
of certain tumour types. Other forms of therapy such as surgery, are known. However  
it is clear that novel approaches and entities for cancer therapies are required if  
20 significant progress in the clinical management of this disease is to be achieved.

Targetted enzyme prodrug therapies may offer significant improvements in cancer  
therapy, either alone or in combination with existing treatment regimes. One such  
therapy relates to the use of molecular chimaeras, which encode a heterologous  
25 enzyme and, which are delivered to targetted cells. Intracellular expression of the  
enzyme allows catalysis of a subsequently administered prodrug to its active cytotoxic  
or cytostatic form. The therapy is known as gene or virus directed enzyme prodrug  
therapy (GDEPT or VDEPT).

WO-A-90 07936 describes a treatment for an infection or a hyperproliferative disorder  
30 which is characterised by the presence, in the affected cells, of a trans-acting factor  
capable of regulating gene expression by inserting into the cells a polynucleotide  
construct having a cis-acting regulatory sequence which is regulated by the trans-  
acting factor and an effector gene which renders said cell susceptible to protection or  
destruction. For example, the cis-acting region may be homologous to the HIV tar  
35 region, and the effector gene may encode ricin A or HSV-1 thymidine kinase. Upon

infection with HIV, the HIV tat protein activates the tar region, and induces transcription and expression of ricin A, resulting in cell death, or of HSV-1 tk, resulting in cell death upon treatment with dideoxynucleoside agents such as acyclovir and gancyclovir.

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EP-A-0 334 301 describes methods for the delivery of vectors using recombinant retrovirus wherein the vector construct directs the expression of a protein that activates a compound with little or no cytotoxicity into a toxic product in the presence of a pathogenic agent, thereby effecting localised therapy to the pathogenic agent.

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EP-A-0 415 731 describes molecular chimaeras for use with prodrugs, comprising transcriptional regulatory DNA sequences capable of being selectively activated in a mammalian cell, a DNA sequence operatively linked to the transcriptional regulatory DNA sequence and encoding a heterologous enzyme capable of catalysing the conversion of the prodrug into an agent toxic to the cell.

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EP-0 382 411 proposes a targetted enzyme prodrug therapy for neoplastic disease which utilises antibodies to direct an enzyme such as  $\beta$ -lactamase to neoplastic tissues where the enzyme can catalyse conversion of a prodrug to a cytotoxic agent.

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However, this approach is limited by the availability of suitable targeting antibodies and their lack of ability to penetrate solid tumours and by the fact that the cytotoxic agent is generated extracellularly and therefore must enter the neoplastic cell to exert its cytotoxic effect.

The range of prodrugs suitable for use in GDEPT, and hence the range of cytotoxic or other therapeutic agents which can be targetted using this approach, is limited by the availability of DNA encoding an enzyme which possesses appropriate catalytic activity to convert the prodrug into the active cytotoxic or other therapeutic agent, which DNA is capable of expression in a eukaryote. An object of the present invention is to improve and extend the range of prodrugs which can be used in GDEPT.

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The present invention relates to the use of a  $\beta$ -lactamase enzyme in GDEPT. More particularly the present invention provides a molecular chimaera for use in therapy with a prodrug, the chimeara comprising a transcriptional regulatory DNA sequence capable of being activated in a targetted mammalian cell and a DNA coding sequence operatively linked to the transcriptional regulatory DNA sequence and encoding a  $\beta$ -

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lactamase enzyme such that on expression of said coding sequence in the targetted cell, the  $\beta$ -lactamase enzyme is capable of catalysing conversion of the prodrug into an agent toxic to the targetted cell.

5       The enzyme  $\beta$ -lactamase has particular advantages for use in GDEPT in terms of the range of toxic agents which can be presented in the form of prodrugs capable of conversion to the active agent by means of the enzyme. In principle any toxic agent can be converted to such a prodrug by conjugation with another compound through a bond capable of being cleaved by  $\beta$ -lactamase. According to one particularly  
10       advantageous embodiment, conjugates are formed between the toxic agent and a cephalosporin. Specific examples of toxic agents include 5-fluorouracil, methotrexate and adriamycin which may be linked in each case to, for example, a cephalosporin (see WO-A-94 01 137 and EP-A-0 382 411) or cephalosporin mustards (see EP-A-0 484 870). In each case the cephalosporin/toxic agent conjugate shows markedly  
15       reduced toxicity but can be converted to the active form by  $\beta$ -lactamase thus making it suitable for use as a prodrug in GDEPT. Other toxic agents can be linked to cephalosporins in a similar way.

20       Prodrugs for use according to the present invention may thus be based on any compound showing a suitable chemotherapeutic effect. Such chemotherapeutic agents are preferably anti-inflammatory, anti-viral or anti-cancer compounds, and more preferably cytotoxic compounds such as nitrogen mustard agents, antifolates, nucleoside analogs, the vinca alkaloids, the anthracyclines, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, the  
25       podophytophyllotoxins, the sulfonylureas (as described in EP-A-0 222,475) and low-molecular-weight toxins such as the trichothecenes and the colchicines. Particularly including doxorubicin, daunorubicin, aminopterin, methotrexate, taxol, methopterin, dichloromethotrexate, mitomycin C, porfirimycin, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, podophyllotoxin, etoposide, melphalan, vinblastine, vincristine,  
30       desacetylvinblastine hydrazide, leurosine, vindesine, leurosine, trichothecene and desacetylcolchicine.

35       The molecular chimera according to the present invention may provide intracellular expression or membrane bound expression or secretion of the  $\beta$ -lactamase enzyme in the targetted cell. Secretion or membrane bound expression of the  $\beta$ -lactamase

enzyme has the advantage of increasing the phenomenon known as "neighbouring cell kill". GDEPT and VDEPT regimes which involve the intracellular expression of the heterologous enzyme and intracellular catalysis of the prodrug are limited in terms of their efficiency in that, for example in tumour therapy using retroviral mediated  
5     targetting, only between 1 and 10% of cells of the solid tumour may be infected by retrovirus. Hence therapy is limited to those 1 to 10% of infected cells and the neighbouring 90-99% of cells remain untreated. Clearly in a hyperproliferative condition it is desirable to maximise cell kill and so the ability to kill uninfected  
10    neighbouring cells is extremely important. Extracellular conversion of the prodrug into the toxic agent can be achieved by secretion or membrane bound expression of the  $\beta$ -lactamase enzyme and active drug may then diffuse and exert its effect in neighbouring cells. This so-called "neighbouring cell kill" can provide a significant increase in the therapeutic effect.

15    To be capable of secretion through the cell membrane, an enzyme should possess a signal sequence at the amino terminus either because it is a secreted enzyme with a naturally occurring signal sequence or because the chimaera expressing the enzyme has been engineered such that the expressed enzyme has an additional amino acid sequence which possesses the properties of a signal sequence. Such signal  
20    sequences are well known in the art and include those indicated or derivable from Nothwehr *et al*, J. Biol. Chem., 265, 21797-21803 (1990), Nothwehr and Gordon, J. Biol. Chem., 265 17202-17208 (1990) and Kohara *et al*, FEBS Letters, 311, 226-230 (1992) and the references contained therein.  
In order to achieve membrane bound expression of the  $\beta$ -lactamase enzyme, the  
25    molecular chimaera should also include at a suitable position DNA sequence encoding a membrane anchoring peptide. Such peptides may be derived, for example, from proteins with C-terminal domains substituted with phospholipid anchors. Such proteins include Thy-1 (Low *et al*, Nature, 318, 62 (1985) and Tse *et al*, Science, 230, 1003 (1985)), the variant surface glycoproteins (VSGs) of African trypanosomes  
30    (Ferguson *et al*, J. Biol. Chem., 260, 1457 (1985)), acetylcholinesterase (Futerman *et al*, Biochem. J., 226, 369 (1985)), 5'-nucleotidase (Low *et al*, Biochim. Biophys. Acta, 508, 565 (1978)) as well as DAF (Davitz *et al*, J. Exp. Med., 163, 1150 (1986) and Medof *et al*, Biochemistry, 25, 6740 (1986)) or proteins known to contain glycopospholipid anchors including acetylcholinesterase (Schumacher *et al*, Nature,  
35    319, 407-409 (1986)), Thy-1 (Seki *et al*, Nature, 313, 485-487 (1985), Moriuchi *et al*,

FEBS Letters, 178, 105-108 (1985)), VSG (*T. brucei*) (Cross *et al*, Philos. Trans. R. Soc. London, Ser. B, 307, 3-12 (1984)) and alkaline phosphatase (Weiss *et al*, Proc. Natl. Acad. Sci. USA, 83, 7182-7186 (1986)). For general reviews on such polypeptides see Low, Biochem. J., 244, 1-13 (1986) and Low *et al*, TIBS, 11, 212-215 (1986).

In the molecular chimaera according to the invention, the coding sequence is under the control of a transcriptional regulatory sequence (TRS) comprising at least a promoter and preferably an enhancer, each of which may either be capable of non-specific expression independent of the type of cell in which expression is occurring or may exhibit a selectivity of expression dependent upon the cellular environment. Preferred TRSs are non-specific, potent promoter/enhancer combinations such as cytomegalovirus promoter/enhancer, SV40 promoter/enhancer and retroviral long terminal repeat promoter/enhancer. Other preferred TRSs include those of  $\beta$ -actin, glyceraldehyde-S-phosphate and tubulin.

Also preferred are TRSs exhibiting cell-type dependent, for example, tissue specific or tumor specific, expression in which case the selection of the TRS, in particular the promoter and enhancer sequence, will depend on the targetted cell type. Examples include the albumin (ALB) and alpha-fetoprotein (AFP) TRS for normal hepatocytes and transformed hepatocytes respectively, the TRS for carcinoembryonic antigen (CEA) for use in transformed cells of the gastrointestinal tract, lung, breast and other tissues: the TRS for tyrosine hydroxylase, choline acetyl transferase or neuron specific enolase for use in neuroblastomas: the TRS for glial fibro acidic protein for use in glioblastomas and the TRS for insulin for use in tumours of the pancreas. Further examples include the TRS specific for gamma-glutamyltranspeptidase for use in certain liver tumours and dopa decarboxylase for use in certain tumours of the lung. The present invention is also useful in treatment of ovarian cancer, colon cancer, breast cancer, prostate cancer and melanomas.

In addition the TRS for certain oncogenes may be used as these are expressed predominantly in certain tumour types. These include the HER-2/neu oncogene TRS which is expressed in breast tumours and the TRS specific for the N-myc oncogene for neuroblastomas.

The ALB and AFP genes exhibit extensive homology with regard to nucleic acid sequence, gene structure, amino acid sequence and protein secondary folding (for review see Ingram *et al* Proc. Natl. Acad. Sci. (USA) 78, 4694-4698 (1981)). These genes are independently but reciprocally expressed in ontogeny. In normal development ALB transcription is initiated shortly before birth and continues throughout adulthood. Transcriptional expression of ALB in the adult is confined to the liver. AFP is normally expressed in foetal liver, the visceral endoderm of the yolk sac and the foetal gastrointestinal tract, but declines to undetectable levels shortly after birth and is not significantly expressed in nonpathogenic or non-regenerating adult liver or in other normal adult tissues. However, AFP transcription in adult liver often increases dramatically in hepatocellular carcinoma (HCC). In addition, transcription may also be elevated in non-seminomatous and fixed carcinoma of the testis: in endodermal sinus tumours in certain teratocarcinomas and in certain gastrointestinal tumours. Liver-specific expression of AFP and ALB is the result of interactions of the regulatory sequences of their genes with trans-activating transcriptional factors found in nuclear extracts from liver. The AFP and ALB TRSs are preferred for generating hepatoma-specific or general liver-specific expression respectively of molecularly combined genes since the AFP and ALB genes are regulated at the transcriptional level and their mRNAs are among the most abundant polymerase II transcripts in the liver.

Several mammalian ALB and AFP promoter and enhancer sequences have been identified (for review see Genes and Develop 1, 268-276 (1987); Science, 235, 53-58 (1987); J. Biol. Chem. 262, 4812-4818 (1987)). These sequences enable the selective and specific expression of genes in liver hepatocytes (normal and transformed) and hepatomas respectively.

Similar to the regulatory structure of the ALB gene the regulatory elements of the AFP genes promote tissue-specific expression in certain liver pathologies, such as HCC (Mol. Cel. Biol. 6, 477-487 (1986); Science, 235, 53-58 (1987)). The regulatory elements of a mammalian AFP gene consist of a specific 5' promoter proximal region (located in some mammalian species between 85 and 52 bp 5' to the gene). This sequence is essential for transcription in hepatomas. In addition there are upstream (5') regulatory elements well defined for the murine AFP gene which behave as classical enhancers (Mol. Cel. Biol. 6, 477-487 (1986); Science, 235, 53-58 (1987)).



These upstream regulatory elements are designated elements I, II and III and are located between 1,000 to 7,600 bp 5' to the transcription initiation site for the AFP murine gene. These three enhancer domains are not functionally equivalent at generating tissue-specific expression of AFP. Elements I and II have the greatest capacity to direct liver-specific expression of AFP. It is important to note that the regulatory sequences of the alpha-fetoprotein gene advantageously contain the sequences not only for tissue-specific transcriptional activation but also for repression of expression in tissues which should not express AFP. In a similar fashion the regulatory regions of the human alpha-fetoprotein gene have been characterised (J. Biol. Chem. 262, 4812-4818 (1987)). A structural gene placed in the correct orientation 3' to the AFP regulatory sequences will enable that structural gene to be selectively expressed in fetal liver hepatomas, non-seminomatous carcinomas of the testis, certain teratocarcinomas, certain gastrointestinal tumours and other normal and pathological tissues which specifically express AFP.

The promoter and enhancer sequences preferably are selected from the TRS for one of albumin (ALB), alphafetoprotein (AFP), carcinoembryonic antigen (CEA) (J. DNA Sequencing and Mapping, Vol 4, 185-196), tyrosine hydroxylase, choline acetyl transferase, neuron-specific enclase, glial fibro acid protein, insulin or gamaglutamytranspeptidase, dopadecarboxylase, HER-2/neu or N-myc oncogene or other suitable genes. Most preferably the TRS for ALB or AFP are used to direct liver specific or hepatoma specific expression respectively.

In preferred embodiments of the present invention the molecular chimaera is selectively expressed in a target cell population. This may be taken to mean that the chimaera is expressed at a higher level in the target than in the non-target cell population and is preferably expressed predominantly or exclusively in that population.

Selective expression may be achieved by inclusion of a target-cell specific TRS (promoter with or without enhancer) as described above or may be a product of the method of delivery of the chimaera to the target cell. Methods capable of providing target cell specific delivery of the chimaera, with subsequent stable integration and expression, include the techniques of calcium phosphate transfection, electroporation, microinjection, liposomal transfer, ballistic barrage or retroviral infection or infection using adenovirus or adeno-associated virus. For a review of this subject see Biotechniques 6(7) (1988).

Such selectivity may be obtained by a variety of such techniques. Physiologically localised delivery of the chimaera for the target cells will reduce the possibility of non-target cells expressing the chimaera. This may be achieved when for example using retroviral or liposome mediated delivery and would involve direct injection to a blood vessel known to supply the target cells. Selectivity may also be obtained using retroviral mediated chimaera delivery in the therapy of hyperproliferative disorders. Retroviruses only infect dividing cells and would therefore only introduce chimaeras to dividing cells. Liposome technology permits the delivery of the chimaera contained therein to be targetted to a particular cell type based on appropriate modifications made to the liposome coat structure. In a preferred embodiment according to the present invention, a number of such methods for obtaining selectivity will be combined to improve the fidelity of selective expression. For example in the treatment of hepatocellular carcinoma, the chimaera may comprise TRSs derived from liver-specific gene promoters such as ALB or AFP, and will be delivered in a retrovirus directly to the hepatic artery. Hence a three-fold level of specificity will be obtained, firstly by the localised delivery which ensures that effectively all the retroviruses infect liver cells, secondly by use of a retrovirus which only infects dividing carcinoma cells and thirdly by liver-specific expression of the TRSs.

One particular method according to the present invention for obtaining selective expression of a molecular chimaera of the present invention delivered using a retrovirus is accomplished by promoting selective infection of liver cells. This technique involves the retroviral env gene present in the packaging cell line which defines the specificity for host infection. The env gene used in constructing the packaging cell line is modified to generate artificial infective virions that selectively infect hepatocytes. As an example a retroviral env gene introduced into the packaging cell may be modified in such a way that the artificial infective virion's envelope glycoprotein selectively infect hepatocytes via the specific receptor mediated binding utilised by the hepatitis B virus (HBV). HBV primarily infects hepatocytes via specific receptor mediated binding. The HBV proteins encoded by the pre-S1 and pre-S2 sequences play a major role in the attachment of HBV to hepatocytes (see *Hepadna Viruses* edited Robinson *et al* 189-203, 205-221 (1987)). The env gene of the packaging cell is modified to include the hepatocyte binding site of the large S HBV envelope protein. Such modifications of the env gene introduced into the packaging

cell may be performed by standard molecular biology techniques well known in the art and will facilitate viral uptake in the target tissue.

5 In the methods of obtaining selective activation which comprise target cell specific delivery systems, the TRS need not be target cell specific and TRSs derived from genes such as  $\beta$ -actin, glyceraldehyde-3-phosphate and cytomegalovirus (e.g. immediate early gene) (see Huber, *et al* Cancer Research, 53, 4619-4626 (1993) and references therein) may be used.

10 The molecular chimaera of the present invention may be made utilising standard recombinant DNA techniques. Thus the coding sequence and polyadenylation signal of for example the cytosine deaminase gene is placed in the proper 3' orientation to the ALB or AFP TRS. These molecular chimaeras enable the selective expression of cytosine deaminase in cells which normally express from ALB or AFP TRSs respectively.

15 As described in more detail below, molecular chimeara have been constructed for secretion, intracellular expression and transmembrane expression of  $\beta$ -lactamase in a mammalian cell. DNA sequences from these constructs are set out in SEQ IDs Nos 3, 5 and 9 respectively.

20 Accordingly there is also provided a method of constructing a molecular chimaera comprising operatively linking a DNA sequence comprising a TRS capable of being activated in a mammalian cell to a DNA sequence encoding a  $\beta$ -lactamase enzyme capable of expression in a mammalian cell.

25 According to one embodiment the present invention provides a molecular chimeara as defined above wherein the sequence providing for intracellular expression of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 5. Preferably the sequence providing for intracellular expression of the  $\beta$ -lactamase enzyme has the sequence of SEQ ID NO 5.

30 According to another embodiment the present invention provides a molecular chimeara as defined above wherein the sequence providing for secretion of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 3. Preferably the sequence providing for secretion of the  $\beta$ -lactamase enzyme has the sequence of  
35 SEQ ID NO 3.

According to a further embodiment the present invention provides a molecular chimera as defined above wherein the sequence providing for membrane bound expression of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 9. Preferably the sequence providing for membrane bound expression of the  $\beta$ -lactamase enzyme has the sequence of SEQ ID NO 9.

The technique of retroviral infection of cells to integrate artificial genes employs retroviral shuttle vectors which are known in the art (see for example Mol. and Cell Biol. 6, 2895-2902 (1986)). Essentially retroviral shuttle vectors are generated using the DNA form of the retrovirus contained in a plasmid. These plasmids also contain sequences necessary for selection and growth in bacteria. Retroviral shuttle vectors are constructed using standard molecular biology techniques well known in the art. Retroviral shuttle vectors have the parental endogenous retroviral genes (e.g. gag, pol and env) removed and the DNA sequence of interest inserted, such as the molecular chimera which have been described. They however contain appropriate retroviral regulatory sequences for viral encapsidation, proviral insertion into the target genome, message splicing, termination and polyadenylation. Retroviral shuttle vectors have been derived from the Moloney murine leukaemia virus (Mo-MLV) but it will be appreciated that other retroviruses can be used such as the closely related Moloney murine sarcoma virus. Certain DNA viruses may also prove to be useful as a delivery system. The bovine papilloma virus (BPV) replicates extrachromosomally so that delivery system based on BPV have the advantage that the delivered gene is maintained in a nonintegrated manner. Adenoviruses and adeno-associated viruses may also be used.

Thus according to a further aspect of the present invention there is provided a retroviral shuttle vector containing a molecular chimera as hereinbefore defined.

The advantages of a retroviral-mediated gene transfer system are the high efficiency of the gene delivery to the targeted tissue sequence specific integration regarding the viral genome (at the 5' and 3' long terminal repeat (LTR) sequences) and little rearrangements of delivered DNA compared to other DNA delivery systems.

Accordingly in a preferred embodiment of the present invention there is provided a retroviral shuttle vector comprising a DNA sequence comprising a 5' viral LTR

sequence, a cis acting psi encapsidation sequence, a molecular chimaera as hereinbefore defined and a 3' viral LTR sequence.

5 In a preferred embodiment and to help eliminate non-target-specific expression of the molecular chimaera, the molecular chimaera is placed in opposite transcriptional orientation to the 5' retroviral LTR. In addition a dominant selectable marker gene may also be included which is transcriptionally driven from the 5' LTR sequence. Such a dominant selectable marker gene may be the bacterial neomycin-resistance gene NEO (aminoglycoside-3-phosphotransferase type II) which confers on eukaryotic cells  
10 resistance to the neomycin analogue G418 sulphate (Geneticin - trade mark). The NEO gene aids in the selection of packaging cells which contain these sequences.

The retroviral vector used may be based on the Moloney murine leukaemia virus but it will be appreciated that other vectors may be used. Such vectors containing a NEO  
15 gene as a selectable marker have been described, for example, the N2 vector (Science, 230, 1395-1398 (1985)).

A theoretical problem associated with retroviral shuttle vectors is the potential of retroviral long terminal repeat (LTR) regulatory sequences transcriptionally activating a  
20 cellular oncogene at the site of integration in the host genome. This problem may be diminished by creating SIN vectors. SIN vectors are self-inactivating vectors which contain a deletion comprising the promoter and enhancer regions in the retroviral LTR.

The LTR sequences of SIN vectors do not transcriptionally activate 5 or 3 genomic sequences. The transcriptional inactivation of the viral LTR sequences diminishes  
25 insertional activation of adjacent target cell DNA sequences and also aids in the selected expression of the delivered molecular chimaera SIN vectors are created by removal of approximately 299 bp in the 3 viral LTR sequence (Biotechniques, 4, 504-512 (1986)).

30 Thus preferably the retroviral shuttle vector of the present invention are SIN vectors. Since the parental retroviral gag pol and env genes have been removed from these shuttle vectors a helper virus system may be utilised to provide the gag pol and env retroviral gene products trans to package or encapsidate the retroviral vector into an infective virion. This is accomplished by utilising specialised "packaging" cell lines  
35 which are capable of generating infectious synthetic virus yet are deficient in the ability

to produce any detectable wild-type virus. In this way the artificial synthetic virus contains a chimaera of the present invention packaged into synthetic artificial infectious virions free of wild-type helper virus. This is based on the fact that the helper virus that is stably integrated into the packaging cell contains the viral structural genes but is lacking the psi site and cis acting regulatory sequence which must be  
5 contained in the viral genomic RNA molecule for it to be encapsidated into an infectious viral particle.

Accordingly the present invention provides an infective virion comprising a retroviral shuttle vector as hereinbefore described said vector being encapsidated within viral  
10 proteins to create an artificial infective replication-defective retrovirus.

In addition to removal of the psi site additional alterations can be made to the helper virus LTR regulatory sequences to ensure that the helper virus is not packaged in  
15 virions and is blocked at the level of reverse transcription and viral integration.

Alternatively helper virus structural genes (i.e. gag pol and env) may be individually and independently transferred into the packaging cell line. Since these viral structural genes are separated within the genome of the packaging cell, there is little chance of  
20 covert recombinations generating wild-type virus.

In a further aspect of the present invention there is provided a method for producing infective virions of the present invention by delivering the artificial retroviral shuttle vector comprising a molecular chimaera of the invention as hereinbefore described  
25 into a packaging cell line.

The packaging cell line may have stably integrated within it a helper virus lacking a psi site and other regulatory sequence as hereinbefore described or alternatively the packaging cell line may be engineered so as to contain helper virus structural genes  
30 within its genome.

The present invention further provides an infective virion as hereinbefore described for use in therapy particularly for use in the treatment of cancer and more particularly for use in the treatment of hepatocellular carcinoma, non-seminomatous carcinoma of the  
35 testis, certain teratocarcinomas and certain gastrointestinal tumours.

The infective virion according to the invention may be formulated by techniques well known in the art and may be presented as a formulation with a pharmaceutically acceptable carrier therefor. Pharmaceutical acceptable carriers in this instance may comprise a liquid medium suitable for use as vehicles to introduce the infective virion into the patient. An example of such a carrier is saline. The infective virion may be a solution or suspension in such a vehicle. Stabilisers and antioxidants and or other excipients may also be present in such pharmaceutical formulations which may be administered to a mammal by any conventional method e.g. oral or parenteral routes. In particular the infective virion may be administered by intra-venous or intra-arterial infusion. In the case of treating HCC intra-hepatic arterial infusion may be advantageous.

Accordingly the invention also provides pharmaceutical formulations comprising a molecular chimaera of the present invention contained within one of, an infective virion or a liposome or a packaging cell mix, in admixture with a pharmaceutically acceptable carrier, and pharmaceutical formulations comprising a molecular chimaera virion, vector, liposome or packaging cell mix of the present invention in admixture with a pharmaceutically acceptable carrier.

Additionally the present invention provides methods of making pharmaceutical formulations as herein described comprising mixing an artificial infective virion containing a molecular chimaera with a pharmaceutically acceptable carrier.

The invention also includes the use of any molecular chimaera, vector, virion, liposome or pharmaceutical formulation of the present invention in human therapy and in the manufacture of a medicament for use in the treatment of pathological states.

The invention also includes methods of medical therapy comprising the use of any molecular chimaera, vector, virion, liposome or pharmaceutical formulation of the present invention.

Also included within the scope of the present invention is a protein encoded by a molecular chimaera of the present invention and any combination of such a protein and a prodrug which can be catalysed by the enzyme component of that protein.

5 The precise dosage to be administered to a patient will ultimately be dependent upon the discretion and professional judgement of the attendant physician and will be a product of the particular targetting mechanism chosen. References contained herein to the efficiency of targetting of retroviruses, liposome etc. may be used to determine appropriate dosage levels.

10 The amounts and precise regime in treating a mammal, will of course depend on a number of factors including the type and severity of the condition to be treated. However, for hepatocellular carcinoma an intrahepatic arterial infusion of the artificial infective virion at a titre of between  $2 \times 10^5$  and  $2 \times 10^7$ , for example  $5 \times 10^5$ ,  $8 \times 10^5$ ,  $2 \times 10^6$ ,  $5 \times 10^6$  or  $8 \times 10^6$ , colony forming units per ml (CFU/ml) infective virions is likely to be suitable for a typical tumour. Total amount of virions infused will be dependent on tumor size and would probably be given in divided doses.

15 The dose of prodrug will advantageously be in the range of 0.1 to 250mg per kilogram body weight of recipient per day, preferably 0.1 to 100 mg per kilogram bodyweight.

20 The present invention further provides a method of treating cancer, a viral infection or an inflammatory condition which comprises administering an effective amount of chimera, vector, packaging cell line or infective virion according to the invention.

The viral infection includes, for example, HIV, HBC, HCV and herpes family virus.

25 The invention is illustrated by the following examples in which reference is made to the accompanying drawings. In the drawings:

Figure 1 shows cellular location of  $\beta$ -lactamase activity in mammalian cells transfected with  $\beta$ -lactamase constructs;

30

#### Example

##### (i) Cloning of *E. coli* $\beta$ -Lactamase for Human Cell Expression

35 We have constructed unique DNA constructs containing the bacterial  $\beta$ -lactamase gene which, when delivered to human cells, result in expression of functional  $\beta$ -



lactamase. The advantages of  $\beta$ -lactamase as a prodrug activating enzyme are 1) the enzyme is kinetically very efficient and 2) because of a unique activation mechanism, a prodrug of virtually any drug can be made as an efficient substrate for the enzyme. The implications of this to cancer therapy is that it permits the use of combination prodrug therapy to counter resistance phenomena as well as allows one to choose drugs appropriate to the tumor target. For example, to target lung cancer, prodrugs of methotrexate (5798W93) and 5-fluorouracil (1614W94) have been synthesized.  $\beta$ -lactamase constructs have been created which give rise to secreted, intracellular and membrane-anchored forms.

#### (ii) Construction of Secretory $\beta$ -lactamase Constructs

To create a DNA construct which would express secretory  $\beta$ -lactamase in human cells, the coding region of TEM  $\beta$ -lactamase (Sykes and Matthews, *J. Antimicrob. Chemo.*, 2, 115-157 (1976); Ambler and Scott, *Proc. Natl. Acad. Sci. USA*, 75, 3732-3736 (1978)) was used. Since it exists in the periplasm of bacteria, the unmodified coding region of TEM  $\beta$ -lactamase contains a signal peptide (Sutcliffe, *Proc. Natl. Acad. Sci. USA*, 75, 3737-3741 (1978)). Sequences useful for the cloning and expression of this gene in a eukaryote were added to flanking sequence during PCR by including the sequences in the PCR primers. The sequence of the forward primer (JM1) was 5'-TTGCATAAGCTTGCCACCATGAGTATTCAACATTTCCGTGTC (42-mer) (SEQ ID NO 1). The sequence of the reverse primer (JM2) was 5'-GATCTGTCTAGATTACCAATGCTTAATCAGTGAGGC (36-mer) (SEQ ID NO 2). The forward primer contains a Hind III restriction site (AAGCTT) for subsequent cloning of the PCR product, and a sequence (GCCACC) which confers optimal translation efficiency in vertebrates (Kozak, *J. Cell Biol.* 115, 887-903 (1991)) immediately 5-prime to the initiator methionine codon (ATG) of the  $\beta$ -lactamase coding region. The reverse primer contains an Xba I restriction site (TCTAGA) adjacent to the stop codon (TAA) of the  $\beta$ -lactamase coding region.

The PCR reaction was carried out for 25 cycles using standard conditions and using Vent DNA Polymerase (New England Biolabs, Inc., Beverly, MA, USA) in 4 mM  $\text{MgSO}_4$  and 200  $\mu\text{M}$  of each dNTP and 1 pmol/ $\mu\text{l}$  forward and reverse primers. PCR thermal cycling conditions were 95°C, 1 min; 60°C, 1 min; 75°C, 1 min. 25 cycles then 75°C, 5 min. The approximately 800 base pair PCR product was gel-purified using the

Glass-Max kit (Life Technologies, Inc., Gaithersburg, MD, USA). The purified PCR product was restriction digested with Hind III and Xba I, re-purified by gel electrophoresis, and ligated into the multiple cloning site of the pRc/CMV vector (InVitrogen, Inc., San Diego, CA). The orientation of the  $\beta$ -lactamase insert in this  
5 vector places the  $\beta$ -lactamase gene under the transcriptional regulation of the intermediate/early CMV promoter as well as followed a bovine growth hormone poly(A) addition signal. The sequence of the construct (designated pCMV-BL) is shown in SEQ ID NO 3 along with the amino acid sequence of inserted secretory  $\beta$ -lactamase.

#### 10 (iii) Construction of Intracellular $\beta$ -lactamase Constructs

To create a DNA construct for expression of intracellular  $\beta$ -lactamase in human, modifications to the terminus of the  $\beta$ -lactamase gene in pCMV-BL were carried out using PCR. The forward primer (JM30) for these reactions consisted of the sequence  
15 5'-TTGCATAAGCTTGCCACCATGCACCCAGAAACGCTGGTG (39-mer) (SEQ ID NO 4). This forward primer consists of a Hind III restriction site (AAGCTT), a consensus site for optimal translation efficiency (GCCACC) in vertebrates (Kozak, 1991 *supra*) and an ATG initiator codon immediately adjacent to the sequence representing the mature amino-terminus of TEM  $\beta$ -lactamase (Sutcliffe, 1978 *supra*). When used in a  
20 PCR reaction in combination with the JM2 reverse primer described above, the resulting PCR product would contain a deleted signal peptide and a new initiator methionine codon adjacent to the mature coding region of  $\beta$ -lactamase. This PCR reaction was carried out using PCR conditions identical to those described for pCMV-BL, except that JM30 was substituted for JM1.

25 The approximately 700 base pair PCR product was gel-purified using the Glass-Max kit (Life Technologies, Inc., Gaithersburg, MD, USA). The purified PCR product was restriction digested with Hind III and Xba I, repurified by gel electrophoresis, and ligated into the multiple cloning site of the pRc/CMV vector (InVitrogen, Inc., San  
30 Diego, CA, USA) as described above for pCMV-BL. The sequence of the construct (designated pCMV- $\Delta$ BL) is shown in SEQ ID NO 5 along with the amino acid sequence of inserted intracellular  $\beta$ -lactamase.

#### (iv) Construction of Membrane-Bound $\beta$ -lactamase Constructs

A membrane-bound form of  $\beta$ -lactamase would be useful in prodrug therapies since the enzyme is active and does not diffuse from the site of expression and since the external activation of prodrug guarantees bystander effects of the activated drug. This chimeric enzyme may also have potential as a potent immunostimulatory molecule since the membrane location of the protein may enhance its presentation on MHC Class II molecules.

To create a DNA construct which would express  $\beta$ -lactamase inserted in the external portion of the cell membrane in human, a membrane-spanning domain was appended to the carboxy-terminus of the secretory  $\beta$ -lactamase coding region contained in pCMV-BL. The membrane sequence was derived from the human C mu IgM heavy chain gene (Dorai, Nucl. Acids Res., 17, 6412 (1989)). This was done by fusing a 300 base pair sequence representing the human IgM membrane-spanning domain (from plasmid IgM/TM/PCRII which contains exons M1 and M2 separated by a single intervening sequence) in-frame to the carboxy-terminus of the secretory  $\beta$ -lactamase gene.

The first step in this process was to delete the termination codon in the  $\beta$ -lactamase sequence contained in pCMV-BL. This was done by PCR amplification of the insert using the forward primer JM1 (see above) in combination with the reverse primer MEM1. MEM1 consists of the sequence  
5'-TGACAATCTAGAGAGGGGGAGGTGAGCGCCGACGAG (36-mer) (SEQ ID NO 6). MEM1 contains sequence representing the carboxy-terminus of secretory  $\beta$ -lactamase excepting the translation termination signal (TAA) which is replaced by an Xba I restriction site. The hexameric Xba I sequence is in-frame with the coding region of  $\beta$ -lactamase and represents a Ser-Arg amino acid sequence. This PCR product was amplified as described above, gel-purified, and cloned into the Hind III and Xba I sites of pRc-CMV. This plasmid was designated pCMV-MEM1.

To attach a carboxy-terminal membrane spanning domain, a 300 base-pair sequence from plgM/TM/PCRII was amplified with oligos MEM2 and MEM3. MEM2 consists of the sequence  
5'-TGACAATCTAGAGAGGGGGAGGTGAGCGCCGACGAG (36-mer) (SEQ ID NO 7). MEM3 consists of the sequence

5'-TGACAAGGGCCCCTCTGGTCTCCGATGTTCTTC (33-mer) (SEQ ID NO 8). MEM2 represents the amino-terminus of the IgM trans-membrane domain (beginning at nucleotide 489; GenBank Accession #X14939) flanked by an Xba I restriction site (TCTAGA). MEM3 represents the carboxy-terminus of the trans-membrane domain (ending at nucleotide 815; GenBank Accession #X14939) flanked by an Apa I restriction site (GGGCCC). These oligos were used to carry out PCR as described above and the approximately 300 base-pair product was restriction digested, gel-purified, and cloned into the Xba I and Apa I sites of pCMV-MEM1. The sequence of the construct (designated pCMV-BLIgM) along with the amino acid sequence of inserted membrane-anchored  $\beta$ -lactamase is shown in SEQ ID NO 9.

**(v) Determination of Cellular Locations of Targeted  $\beta$ -Lactamase Protein**

Confirmation of the predicted locations of each of the  $\beta$ -lactamase constructs was carried out using transient DNA transfections in a mammalian cell line. Transfections were carried out by liposome-mediated DNA delivery using lipofectamine (Life Technologies, Inc., Gaithersburg, MD, USA). Experiments were performed according to manufacturer's instructions, varying the number of cells, amount of transfection reagent, and amount of DNA to determine optimum conditions. Typically, 60 x 15mm tissue culture plates containing approximately  $3 \times 10^5$  to  $1 \times 10^6$  cells were employed. After transfections using either pCMV-BL, pCMV-dBL, or pCMV-BLIgM, transfected cells were resuspended in 50 mM Tris-Cl (pH 7.4), 0.1 mM EDTA containing PMSF and leupeptin, swollen on ice for 10 min, then lysed using a Dounce homogenizer. After centrifugation at 800 x g for 6 min, supernatant (cytosolic fraction) was recentrifuged at 30 psi for 20 minutes in a Beckman AirFuge. Pellets from both centrifugations (which include membranes and nuclei) were combined. Each fraction was assayed for activity using the chromogenic substrate PADAC, added to a final concentration of 20 mM (Calbiochem, Corp.). Absorbance at 570 nm was measured using the auto-rate assay of a Kontron Model 9310 spectrophotometer. To assess secreted  $\beta$ -lactamase levels, PADAC assays were carried out on the cell-free media after transfections.  $\beta$ -lactamase enzyme activity was measured using PADAC (-Calbiochem, Corp.) which serves as a chromogenic substrate of  $\beta$ -lactamase activity (Schindler and Huber, *Enzyme Inhibitors*, Brodbede, Ed., pp 169-176, Verlag Chemie, Weinheim (1980). A 500  $\mu$ M PADAC stock was made in water, filtered through a 0.22  $\mu$ m filter, and added to media to give a final concentration of 20  $\mu$ M. Decreases in

absorbance at 570 nm were measured using the auto-rate assay of a Kontron UV/VIS spectrophotometer.

5 The data in Figure 1 show that at 48 hours after transfection with lipofectamine, large amounts of  $\beta$ -lactamase are secreted from cells transfected with pCMV-BL. The cellular activity seen with this construct is presumably the enzyme contained in secretory granules in the process of being exported. In contrast the activity seen using pCMV- $\Delta$ BL is completely localized to the cellular fraction. Based on the magnitude of this activity, we estimate that the enzyme from the secretory  $\beta$ -lactamase  
10 construct represents 5-10% of total cellular protein made per 24 hours per cell. The activity measured using the membrane construct was found almost exclusively in the membrane fractions.

15 In order to characterize the polarity of the active membrane form of  $\beta$ -lactamase, whole cell assays were carried out. Transient transfections of human lung adenocarcinoma with pCMV-BLIgM were carried out.  $\beta$ -Lactamase activity was detected only if the assay media was in contact with the cells, indicating that the enzyme must be membrane-bound located on the exterior face of the membrane. Activity was not detected using the same method when a stable cell line expressing  
20 the intracellular form of  $\beta$ -lactamase was used as a control, indicating that the substrate does not penetrate cells.

To further confirm the localization of the membrane-form of  $\beta$ -lactamase, stable lines were generated for use in immunohistochemistry experiments. To create stable lines,  
25 large-scale transfections in A549 cells were performed. Since pCMV-BL, pCMV- $\Delta$ BL, and pCMV-BLIgM contain the neomycin<sup>R</sup> gene, stable lines could be selected after passaging the lines in media containing the antibiotic, G418. Clonal lines were derived which secrete  $\beta$ -lactamase (pCMV-BL/A549), lines which synthesize an intracellular  $\beta$ -lactamase (pCMV- $\Delta$ BL/A549), and lines which synthesize membrane-bound  $\beta$ -lactamase (pCMV-BLIgM/A549). Cells from each clone were used for  
30 immunohistochemistry using a primary rabbit anti- $\beta$ -lactamase antibody followed by a fluorescein-labeled secondary goat anti-rabbit antibody. In this test, cells were not fixed prior to treating with the antibodies. Only the stable line expressing membrane-bound  $\beta$ -lactamase displayed fluorescent labeling above background levels (data not  
35 shown).

**(vi)  $\beta$ -Lactamase Delivery to Cells Confers Sensitivity to Cephalosporin Prodrugs**

**A.  $\beta$ -Lactamase efficiently activates 5798W93 and 1614W94**

- 5 Prodrugs of methotrexate (5798W93) and 5-fluorouracil (1614W94) represent the parent drugs linked to cephalothin. The kinetic parameters of prodrug activation were measured by incubating various concentrations of prodrug with purified  $\beta$ -lactamase followed by HPLC analysis to determine the rate of prodrug conversion.  $\beta$ -Lactamase efficiently activates both 5798W93 and 1614W94 with a  $k_{cat}/K_M$  (specificity constant) of 10 272 and 67  $\text{sec}^{-1} \text{mM}^{-1}$ , respectively.

**B. Combination of the  $\beta$ -Lactamase Gene with 5798W93 and 1614W94 Confers Toxicity**

- 15 We have evaluated the *in vitro* toxicity of the  $\beta$ -lactamase prodrugs in the presence and absence of the  $\beta$ -lactamase gene. Cytotoxicity was quantitated by determining  $\text{IC}_{50}$ s in treated A549 human lung adenocarcinoma cells using an SRB-based growth inhibition assay (Nair et al., J. Med. Chem., 32, 1277-1279 (1989)).

- 20 In the absence of the  $\beta$ -lactamase gene, methotrexate was 10-fold more toxic than the methotrexate prodrug 5798W93, and fluorouracil was 20-fold more toxic than the fluorouracil prodrug 1614W94 (Table 1). When A549 cells which contained stable integrated copie(s) of the secretory  $\beta$ -lactamase gene (A549-BL) were tested, methotrexate and its prodrug 5798W93 were equally toxic (Table 1). This experiment implies that the delivery of the  $\beta$ -lactamase gene to tumor cells will make them 25 sensitive to cephalosporin prodrugs.

- 30 The relatively small differential between the toxicity of methotrexate and 5-fluorouracil and their respective prodrugs in the absence of the  $\beta$ -lactamase gene was unexpected. This is because, for both parent drugs, the mechanism of action is well understood and the chemical modification made by attaching cephalothin to these compounds should clearly detoxify the drugs. For example, transport of methotrexate into cells depends on availability of the terminal glutamate moiety which is blocked in 5798W93. Toxicity of 5-fluorouracil depends on the availability of the N1 group since this group is necessary for glycosidic bond formation and concomitant nucleoside 35 formation. The N1 group is blocked in 1614W94. It is clear that the observed toxicity

of these prodrugs *in vitro* reflects some degree of chemical instability of the prodrugs which could result in significant breakdown of the prodrugs during the 72-hour incubation utilized in the  $IC_{50}$  determination.

5 Support for this notion comes from two lines of evidence. The first is that no toxicity is observed when either prodrug is given to mice at a dose equivalent to an  $LD_{100}$  for the parental drug. The lack of toxicity in these cases is explained by the relatively short half life of the drug *in vivo* ( $t_{1/2} \approx 20$  minutes) in contrast to the exposure of cells to the prodrug for 72 hours *in vitro*.

10

The second line of evidence is shown by direct measurement of *in vitro* toxicity by short-term assays (3 hour exposure of cells to prodrug). Using a sensitive assay for cell toxicity, a 6- $^3H$ -deoxyuridine based assay which measures inhibition of thymidylate synthase and DNA synthesis, we could measure toxicity over time as short as a three hour interval. During this shorter interval, the differential between prodrug and parent drug increased significantly (Table 2). These data are consistent with the idea that the prodrug toxicities reported in Table 1 result from chemical instability of the prodrugs over the long time-course (72 hours) of those experiments.

15

20 **(vi) Antitumor Evaluation of Secretory  $\beta$ -Lactamase *in vivo* Using Liposome-Mediated DNA Delivery**

Secretory  $\beta$ -lactamase and cytosine deaminase DNA constructs were compared for antitumour effects in mice bearing subcutaneous (s.c.) A549 human lung adenocarcinoma tumours. Results are shown in Table 3. Plasmid DNA expression vectors encoding either cytosine deaminase (CD) or secretory  $\beta$ -lactamase (BL) under the transcriptional control of the non-specific CMV promoter were encapsidated in cationic liposomes (25 $\mu$ g DNA; 25 nmol liposomes). Mice bearing A549 s.c. tumours were treated with five intratumoral injections of liposomal DNA. Prodrug therapy (1614W94 (50 mg/kg; i.p., qd x 5) or 5-FC (500 mg/kg; i.p., qd x 5) was initiated two days after DNA treatment. Inhibition of tumour growth was determined on day 47. Both CD and BL constructs resulted in similar antitumour activity *in vivo*. 1614W94 administration resulted in about 60% inhibition of tumour growth (Table 3). 5-FC administration resulted in about 70% inhibition of tumour growth, whereas DNA liposomes alone and 5-FU alone (25mg/kg, i.p., qd x 5) resulted in only about 20%

25

30

inhibition of tumour growth (Table 3). Thus, liposomal DNA/5-FU prodrug combinations resulted in s.c. tumour regressions.

5        Secretory  $\beta$ -lactamase and cytosine deaminase DNA constructs were also evaluated by intrathoracic (i.t.) injection of liposomal DNA into the pleural space of mice bearing tumors. Results are shown in Table 4. Mice bearing human large cell lung H460 i.t. tumours received DNA encoding either CD or BL under the transcriptional control of the CMV promoter. DNA was dosed by i.t. injection on days 6, 7, 12 and 13. Prodrugs for the respective enzyme were dosed on days 7-16 (5-FC, 500 mg/kg; 10        1614W94, 70 mg/kg; i.p., qd x 10). Animal survival was evaluated 30 days after tumour implantation. All nontreated mice and mice treated with 5-FU (30 mg/kg i.p., qd x 5) died from tumour by 30 days. CMV-BL/1614W94 treatment increased survival to 60%, and CMV-CD/5-FC treatment also increased the survival to 40% (Table 4).



Table 1 Cytotoxicity (SRB Assay)

		IC <sub>50</sub>	
		A549	A549-BL
5	Methotrexate	10 nM	N.D.
	1 $\mu$ M, 3h		
	5798W93	100 nM	N.D.
	1 $\mu$ M, 3h		
10	5-Fluorouracil	1.9 $\mu$ M	1.4 $\mu$ M
	1 $\mu$ M, 5h		
	1614W94	40 $\mu$ M	1.7 $\mu$ M
	1 $\mu$ M, 5h		

Table 2 Cytotoxicity (6-[<sup>3</sup>H]-Deoxyuridine Assay)

		% Inhibition	
		A549	A549-BL
15	Methotrexate	82 $\pm$ 4	88 $\pm$ 6
	1 $\mu$ M, 3h		
	5798W93	3 $\pm$ 2	38 $\pm$ 3
	1 $\mu$ M, 3h		
20	5-Fluorouracil	95 $\pm$ 8	91 $\pm$ 10
	1 $\mu$ M, 5h		
	1614W94	-2 $\pm$ 3	33 $\pm$ 6
	1 $\mu$ M, 5h		

Table 3

**Antitumour Effects of Secretory  $\beta$ -Lactamase and Cytosine Deaminase Genes in Mice Bearing Subcutaneous A549 Human Lung Adenocarcinoma**

5

Therapy Group	Tumour Volume (mm <sup>3</sup> )	Percentage Inhibition (Relative to Control)
Phosphate Buffered Saline (No DNA)	1268 $\pm$ 212	0
CMV-BL and 1614W94	511 $\pm$ 86	60
CMV-CD and 5-Fluorocytosine	380 $\pm$ 237	70
5-Fluorouracil alone	1021 $\pm$ 37	19

Table 4

**Antitumour Effects of Secretory  $\beta$ -Lactamase and Cytosine Deaminase Genes in Mice Bearing Intrathoracic H460 Human Large Cell Lung Tumours**

5

Therapy Group	Mean Days of Survival	p Value	Increased Life Span (%)
Phosphate Buffered Saline (No DNA)	20	-	-
CMV-CD + PBS	23	0.245	15
CMV-CD + 5-Fluorocytosine	27	0.009	40
CMV-BL + 1614W94	32	0.012	60

10

NOTE: SEQUENCES ARE AS FOLLOWS:

SEQ ID No 1 = JM1      SEQ ID NO 2 = JM2

SEQ ID NO 3 = pCMV-BL   SEQ ID NO 4 = JM30

SEQ ID NO 5 = pCMV- $\Delta$ BL   SEQ ID NO 6 = MEM1

15

SEQ ID NO 7 = MEM2      SEQ ID NO 8 = MEM3

SEQ ID NO 9 = pCMV-BLlgM]

SEQ ID No 3 - PCMV-BL

CGATGTACGGGCCAGATATACGGCTTGACATGGATTATTGACTAGTTATTAAAGTAATCAATTACGGGGTCTTAGTTTCATAGCCCA  
TATATGGAGTTCCGGCTTACATTAATTACGGTAATGGCCCGCTGGCTGACCGCCCAACGACCCCGCCCAATTGACGTCAATTAATGA  
CGTATGTTCCCATAGTAAACGGCAATAGGGACTTTCCATTGAGCTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACA  
TCAAGTGTATCATATGCCAAGTACGGCCCTTATGACGTCAATGACGGTAAATGGCCCGCTGGCATATAGCCAGTACATGACCTTA  
TGGGACTTTCCCTACTTGGCAGTACATCTACGTATTAGTCTGCTATTACCATGGTGAATGGGTTTGGCAGTACATCAATGGGGTG  
GATAGCGGTTTGACTACGGGGATTCCAAAGTCTCCACCCCATTTGACGTCAATGGAGTTTGTTTTGGCACCATAATCAACGGGACTT  
TCCAAAATGTGTAACTCCGCCCATTTGACGCAATGGGGGTAGGCGGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGG  
HindIII (683)  
CTAAGTAGAGAACCCACTGCTTAAGTGGCTTATCGAAATTAATAGCACTCACTATAGGGAGACCGGAGCTTGGCACCATGAGTATTC  
MetSerIleG  
AACATTTCCGCTGCGCCCTTATCCCTTTTGGGGCAATTGGCTTCTGTGTTTGGCTCACCACAGAACGCTGGTGAAGTAAAGA  
InHisPheArgValAlaLeuIleProPhePheAlaAlaPheCysLeuProValPheAlaHisProGluThrLeuValLysValLysAs  
Xmr  
TGCTGAAGTCAGTTGGGTGACGAGTGGGTTCATCGAACTGGATCTCACAGCGGTAGATCCTTGAGAGTTTTCGCCCGGAGAA  
pAlaGluAspGluLeuGlyAlaArgValGlyTyrIleGluLeuAspLeuAsnSerGlyLysIleLeuGluSerPheArgProGluGlu  
CGTTTTCCTAGTACGCACTTTTAAGTTCTGCTATGTGGCGGGTATATCCCGTATGACCGCGGGCAAGACACTCGGTGGCC  
ArgPheProMetMetSerThrPheLysValLeuLeuCysGlyAlaValLeuSerArgIleAspAlaGlyGlnGluLeuGlyArgA  
ScaI (999)  
GCATACACTATCTCAGATTGACTTGGTTGACTACTCACCACTCAGAAAGCATCTTACGGATGGCATGACAGTAAAGAAATTATG  
rgIleHisTyrSerGlnAsnAspLeuValGluTyrSerProValThrGluLysHisLeuThrAspGlyMetThrValArgGluLeuCy  
PvuI (1110)  
CAGTGCTGCCATACCAAGAGTGAATACACTGGGCCCACTTACTTCTGACAAAGATCGGAGGACCGAAGGAGCTAACCGCTTTTTCG  
sSerAlaAlaIleThrMetSerAspAsnThrAlaAlaAsnLeuLeuThrThrIleGlyGlyProLysGluLeuThrAlaPheLeu  
CACAACTGGGGATCATGTAAGTCCCTTGTGCTGGGACCGGAGCTGAATGAAGCCATACCAAAAGACGAGCGTGACACCAAGA  
HisAsnMetGlyAspHisValThrArgLeuAspArgTrpGluProGluLeuAsnGluAlaIleProAsnAspGluArgAspThrThrM  
TGCTGTAGCAATGGCAACAAGTTGCGCAACTATTAACTGGCAACTACTTACTCTAGCTTCCCGCAACAATTATAGACTGGAT  
etProValAlaMetAlaThrThrLeuArgLysLeuLeuThrGlyGluLeuLeuThrLeuAlaSerArgGlnGlnLeuIleAspTrpMe  
GGAGGCGGATTAAGTTGAGGACCACTTCTGGCTGGCCCTTCCGGCTGGCTGGTTTATGCTGATAAATCTGGAGCCGGTGAGCGT  
tGluAlaAspLysValAlaGlyProLeuLeuArgSerAlaLeuProAlaGlyTrpPheIleAlaAspLysSerGlyAlaGlyGluArg  
GGGTCTGGCGTATCATTCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGAGTCAGGCACTA  
GlySerArgGlyIleIleAlaAlaLeuGlyProAspGlyLysProSerArgIleValValIleTyrThrThrGlySerGlnAlaThrM  
ApaI (1562)  
XbaI (1556)  
TGATGAACGAAATAGACAGATCGCTGAGATAGGTGCTCACTGATTAGCATTTGGTAATCTAGAGGGCCCTATTCATAGTGTACC  
etAspGluArgAsnArgGlnIleAlaGluIleGlyAlaSerLeuIleLysHisTrp...  
TAAATGCTAGAGCTCGCTGATCAGCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTGTTTGGCCCTCCCGGTGCTTCTTGGAC  
CTTGAAGGTGCCACTCCCACTGTCTTTCTTAATAAATGAGGAAATGCATCGCATTTGCTGAGTAGGTGTCAATTCATTTCTGGGG  
PvuII (1)  
GGTGGGGTGGGCGAGGACAGCAAGGGGGAGGATTGGGAAGCAATAGCAGGCATGCTGGGGATGGGTGGGCTCTATGGAACCAAGCTG  
BamHI (1861)  
GGGCTCGAGGGGGGATCCCCACGGCCCTGTAGCGGCGCATTAAGCGCGGGGGTGTGGTGTACGCGCAGCGTGACCGCTACACTT  
GCCAGCGCCCTAGCGCCCGCTCTTTGGCTTTCTTCCCTTCTTCTCGCCAGCTTCCCGGCTTTCCCGGTCAAGCTCTAATTCGGG  
GCATCCCTTTAGGGTTCCGATTAGTGTCTTACGGCACTCGACCCCAAAAACCTTGATTAGGGTGTGGTTCAAGTATGGGCCATC  
GCCCTGATAGACGGTTTTCGGCTTTACTGAGCACTCTTAAATAGTGGACTCTGTTCCAAACCTGGAACAACACTCAACCTTATCTCG  
GTCTATTCTTTGATTTAAGATTTCATCGCCATGTAAAGTGTCAATTAGCATTAATTAATCTTTTATATGCTACTATTCTT  
EcoRI (2321)  
TTGGCTTCGTTACGGGGTGGGTACCGAGCTCGAATTCGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCAAGGCTCCCCAGGC  
AGGCAGAGTATGCAAGCATGCAATTCATTAAGTACAGCAACAGGTGTGGAAAGTCCCAAGGCTCCCAAGCAGGCAGAGTATGCAA  
AGCATGCAATCTCAATTAGTACAGCAACCATAGTCCCGCCCTAATCCCGCCATCCCGCCCTAATCCCGCCCTAATCCCGCCCTAATCC  
CGCCCATGCGTACTAATTTTATTTATTTATGACAGAGGCGAGGCGCCCTCGGCTCTGAGCTATTCCAGAGTATGTAGAGGCTT  
SmaI (2671)  
TTTGGAGGCGCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTT  
CGCATGATTGACAAAGATGGATTGACCGCAGGTCTCCGGCCCTTGGGTGGAGAGGCTATTCGGCTATGACTGGCCACACAGACAA  
TCGGCTGCTCTGATGCGCGGTGTTCGGCTGTACGCGCAGGGGAGCCCGGTCTTTTGTCAAGACCGAATCTGTCGGTGCCTGAA  
PstI (2909)  
PvuII (2964)  
TGAAGTCCAGGACGAGGACGCGCGCTATCGTGGCTGGCCAGCAGGGCGTTCTTCCGACGCTGTGCTCGACGTTGTCACTGAAGCG  
GGAAGGAGCTGGCTGCTATTTGGGCGAAGTCCCGGGCAGGATCTCTGTCACTCTCACTTCTCTGCGGAGAAAGTATCCATATGG  
CTGATGCAAATGCGCGCGCTGCATACGCTTGATCCGGCTACTGCCCATTCGACCAACCAAGCGAAACATCGCATCGAGCGGACGATAC

TCGGATCGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAGAGGCAACAGGGGCTCGCGCCAGCCGACCTGTTCGCCAGGCTCAAG  
GCGCGCATGCCCCGACCGGAGGATCTCGTCGTGACCCATGGCGATGCCCTGCTTGCCTGATATCAATGGTGGAAAATGGCCGCTTTTCCTG  
GATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGCTGATATTCCTGAGAGGCTTGGCGG  
CGAATGGGCTGACCGCTTCCTCGTCTTACGGTATCGCCGCTCCCGATTCCGACGCGCATCGCTTCTATCGCCCTCTTGACGAGTTC  
TTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGAGCCCAACCTGCCATCAGAGATTTCGATTCCACCCCGCCCTTCTA  
TGAAAGGTGGGCTTCGGAATCGTTTTCGGGACGCGCGCTGGATGATCCCTCCAGCGCGGGATCTCATGCTGGAGTTCTTCGCCAC  
CCCAACTTGTTCATTCAGCTTATAATGGTTACAAATAAGCAATAGCATCACAAATTCACAAATAAGCATTTTTTCACTGCAAT

BamHI (3830)

CTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCCCGTCGACCTCGAGAGCTTGGCGTAACTATGGTCATAG  
CTGTTTCTGTGTGAAATTGTATCCGCTCAAAATTCACACAACTACGAGCCGGAAGCATAAAGTGTAAAGCTGGGGTCCCTAAT

PvuII (4029)

GAGTGAGCTAATCACAATTAATTCGGTTGCGCTCACTGCCCCCTTTCCAGTCGGGAAACCTGTCTGCCAGCTGCATTAATGAATCGG  
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CGAGCGGTATCACTCACTCAAGCGGTAATACGGTTATCCACAGAACTAGGGGATACCGCAGGAAGACATGTGAGCAAAAGGCC  
AGCAAAAGGCCAGGAACCTAAAGGCGCGCTTCTGGGCTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAAAATTCGACG  
CTCAAGTCAGAGGTGGCGAACCAGACAGGACTATTAAGATACCAAGCGCTTTCCTCCCTGGAAGCTCCCTCGTGGCTCTCCCTGTTCGG  
AOCCTGCGCGCTTACCGGATACCTGTCCGCTTCTCTCCCTTGGGAAAGCGTGGCGCTTCTCAATGCTCAAGCTGTAGGTATCTCAGTT  
CGGTGTAGGTCTGTTCGCTCCAGCTGGGCTGTGTGCAAGAACCCCGCTTCAGCCCGACCCGCTGGCGCTTATCCGGTAACTATCGTCT  
TGAGTCCACCCCGTAAAGCAGACTTATGCGCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCT  
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GCCGGGAAGCTAGAGTAAGTATTTGCCAGTTAATAGTTTGGCAAGGTATGTGCAATGCTACAGGCATCGTGGTGTCAAGCTCGTC  
GTTTGGTATGGCTTCTATCAGCTCCGGTCCCAAGATCAAGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTAGCTCC

PvuI (5467)

TTCCGTCTCCGTTCGTTGTGAGAGTAAAGTGGCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATTAATCTCTTACTGTCA

ScaI (5578)

TGCCATCCGTAGATGCTTTTCTGTGACTGGTGAATCTCAACCAAGTCATTCTGAGAAATAGTGTATGGGCGACCGAGTTGCTCTTG

XmnI (5695)

CCCGGGTCAATACGGGATAATACCGGCGCACTAGCAGAACTTAAAGTGTCTATCATTTGGAAACGTTCTTCGGGCGGAAACTC  
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TTTCTGGGTGAGCAAAACAGGAGGCAAAATGCCCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCT  
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GTTCCGCGCACHTTCCCCGAAAGTGCACCTGAGTTCAGGATCGGGAGATCTCCCGATCCCTTGTGCTGACTCTCAGTACAAAT  
CTGCTCTGATGCGCATAGTTAAGCCATATCTGCTCCCTGCTGTGTGTGAGGTCCTGAGTAGTGGCGAGCAAAATTAAGT

NruI (6229)

ACAACAAGGCAAGGCTTGACCGCAATTCATGAGAACTGCTTAGGGTTAGGCGTTTTCGCTGCTTCG

SEQ ID No 5 - PCMV- ΔBL

28

GACGGATCGGAGATCTCCCGATCCCTATGGTGGACTCTCAGTACAACTGCTCTGATGCCGCA TAGTTAAGCCAGTATCTGCTCC  
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 NruI (206) SpeI (249)  
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 CGCCCAACGACCCCGCCCATTTGACGTCATAATAGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCATGGG  
 NdeI (483)  
 TGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCATGACGGTA  
 SnaBI (588)  
 AATGGCCCGCTGGCAATTATGCCAGTACATGACCTTATGGGACTTTCTACTTGGCAGTACATCTACGTATTAGTCAATCGCTATTA  
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 HindIII (891)  
 ACTCACTATAGGGAGACCGGAAGCTTGCCACCATGCAACCCAGAAACGCTGGTGAAGTAAGAATGCTGAAGATCAGTTGGGTGCAC  
 MetHisProGluThrLeuValLysValLysAspAlaGluAspGluLeuGlyAla  
 XmnI (1020)  
 GAGTGGGTTACATCGAAGTGGATCTCAACAGCGGTAAGTCTTGAGAGTTTTCGCCCGAAGAACGTTTCCAAATGATGAGCACTT  
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 HeLysValLeuLeuCysGlyAlaValLeuSerArgIleAspAlaGlyGlnGluGluLeuGlyArgArgIleHisTyrSerGlnAsnA  
 Scal (1141)  
 ACTTGGTGTAGTACTCACAGTACAGAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGACGTGCTGCCATAACCATGA  
 spLeuValGluTyrSerProValThrGluLysHisLeuThrAspGlyMetThrValArgGluLeuCysSerAlaAlaIleThrMetS  
 PvuI (1252)  
 GTGATAACACTCGCGGCAACTTACTTCTGACACGATCGGAGGACGAGGAGCTAACCGCTTTTTCGACAAACATGGGGGATCATG  
 erAspAsnThrAlaAlaAsnLeuLeuLeuThrThrIleGlyGlyProLysGluLeuThrAlaPheLeuHisAsnMetGlyAspHis  
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 CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGTTTATGCTGATAATCTGAGCGGTTGAGCGTGGGTCTCGCGGTATCA  
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 ApaI (1704)  
 XbaI (1698)  
 GACAGATCGCTGAGATAGGTGCTCACTGATTAAAGCATTGGTAACTAGAGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCT  
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 CGCTGATCAGCCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTGTTTGGCCCTCCCGGTGCTTCTTTGACCTTGAAGGTGCC  
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 PvuII (1985)  
 CAGGACAGCAAGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAACCACTGGGGCTCGAGGG  
 BamHI (2003)  
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 CTTTGTGATTTATAAGATTTCCATCGCCATGTAAAGTGTACAAATAGCATTAATTACTTCTTTATATGCTACTATTCTTTTGCT  
 EcoRI (2463)  
 TCGTTACCGGGGTGGGTACCGAGCTCGAATTCGTGGAAATGTGTGTCAGTTAGGGTGTGGAAGTCCCCAGGCTCCCCAGGCAGGCA  
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 CCCATGGCTGACTAATTTTTTTTATTTATGACAGAGGCGAGCGCCCTCGGCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTT  
 StuI (2789) SmaI (2813) EcoRV (2825)  
 TTGGAGGCTTAGGCTTTTGCAAAAGCTCCCGGAGCTTGGATATCCATTTTCGGATCTGATCAAGAGACAGGATGAGGATCGTTTC  
 GCATGATTGAACAAGATGGATTGCACGAGGTTCTCCGCCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAA  
 TCGGCTGCTCTGATGCCGCGGTGTCCGGCTGTGAGCGAGGGCGCCCGGTCTTTTGTCAAGACCGACCTGTCCGGTGCCTCGA

PstII (3051)

ATGAACTCAGGACGAGGCGAGCGGGCTATCGTGGCTGGCCACGACGGGCGTTCCCTTGGCAGCTGTGCTCGACGTTGTCACTGAAG  
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 TGGCTGATGCAATGCGGCGGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCGACCAACCAAGCGAAACATCGCATCGAGCGAGCAC  
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 TTTCTGGATTCACTGACATGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGC  
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 CGCCTTCTATGAAGGTTGGGCTTCGGAATCGTTTCCGGGACCGCGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTT  
 CTTCCGCCACCCCACTTGTATTATGACGCTTATAATGGTTACAAATTAAGCAATAGCATCACAAATTCACAAATAAAGCATTTT

PvuII (3106)

BamHI (3972)

TTCACTGCATTCTAGTTGTGGTTTGTCCAACTCATCAATGTATCTTATCATGTCTGGATCCCGTCGACCTCGAGAGCTTGGCGTAA  
 TCATGGTCATAGCTGTTTCCCTGTGTGAAATGTTATCCGCTCACAAATCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCC

PvuII (4)

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 GTCGTTCCGCTCGCGGAGCGGTATCAGCTCACTCAAGGCGGTAAATACGGTTATCCACAGAATCAGGGGATAACGACGGAAGAAC  
 ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCGCGTTGCTGGCGTTTTCATAGGCTCCGCCCCCTGAGGAG  
 CATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCGGACAGGACTATAAGATACAGGCGTTTCCCTCTGAAGCTCCCTC  
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 TCCGCTCCATCCAGTCTATTAAATGTTGGCGGGAAGCTAGAGTAAGTAGTTCCGAGTTAATAGTTTGGCAACGTTGTTGCCATT  
 GCTACAGGCATCGTGGTGTACGCTCGTGGTTGGTATGGCTTCACTCAGCTCCGCTCCCAACGATCAAGGCGAGTTACATGATCC

PvuI (5609)

CCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTATCACTCATGGTT

ScaI (5720)

ATGGCAGCACTGCATAATTCCTTACTGTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCAATTCGA  
 GAATAGTGTATGCGGCGACCGAGTTGCTCTTCCCGGGGTCAATACGGGATAATAACCGCGCCACATAGCAGTACTTTAAAGTGCTC

XmnI (5837)

ATCATTTGGAAAACGTTCTTCCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTGATGTAACCCACTCGTGACCCC  
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 GCGACACGGAATGTTGAATACATCTCTCTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATAC

SEQ ID No 9 - PCMV-BL1gM

GACGGATCGGGAGATCTCCCGATCCCTATGGTCCGACTCTCAGTACAACTGCTCTGATGCCCGCATAGTTAAGCCAGTATCTGCTCC  
 CTGCTTGTGTGTGGAGGTCCGTGAGTAGTCCGCCGAGCAAAATTAAGCTACACACAGCCAGGCTTGACCCGACAAATGCAATGAGA  
 NruI (206) SpeI (249)  
 ATCTGCTTAGGGTTAGGCGTTTTCGCTGCTTCGCGATGTACGGGCGAGATATAGCGGTGACATTGATTTTGTGACTAGTATTAAAT  
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 CGCCCAACGACCCCGCCCATTTGACGTCAATAATGACGTATGTTCCCATAGTACGCCCAATAGGACTTTCCATTGACGTCAATGGG  
 NdeI (483)  
 TGGACTATTTACGGTAATCTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGCGTCAATGCGGTA  
 SmaI (588)  
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 HindIII (891)  
 ACTCACTTAGGGAGACCGGAAGCTTGGCACCATGAGTATTCACATTTCCGTGTCGCCCTTATTCCTTTTTTGGCGCTTTTGGC  
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 XmnI (1086)  
 ATCTCAACAGCGGTAGATCCCTTGAGAGTTTTCGCCCGAAGACGTTTTCCAATGATGAGCACTTTTAAAGTCTGCTATGTGGCG  
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 ScaI (1207)  
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 PvuI (1318)  
 TACTTCTGCAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGACACACATGGGGGATCATGTAATCTGCGCTTGAATGGTGGG  
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 AACCGGATGTAATGAAGCCATACCAACGACGAGCGTGACACACGATGCTGTAGCAATGGCAACACAGTTGGCCAACTATATAA  
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 XbaI (1761)  
 CCTCACTGTTAAGCATTTGGTCTAGAGAGGGGAGGTGAGCGCGGAGGAGGCTTTGAGAACCTGTGGGCCACCGCTCCACCT  
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 hellValLeuPheLeuLeuSerLeuPheTyrSerThrThrValThrLeuPheLys  
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 CGGCTCCCTTCCCGACCAAGGAAGCATTTCACTCTCTGTTGCTGTAGGTGAAATGATCCCAACAGAGACATCGGAGAC  
 ValLys...  
 ApaI (2094)  
 CAGAGGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCCTCGACTGTGCTTCTAGTTGCCAGCCATCTGTT  
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 PvuII (2375) BamHI (2393)  
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 EcoRI (2853)  
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CCATCCCGCCCTTAACCTCCGCGCAGTTCCGCGCATTCCTCGGCCCTTGCGTGAATAATTTTATTTATTCAGAGGGCCGAGGCCG  
 SmaI (3203) EcoRV  
 CCTCGGCTCTTACTATTCAGAGGTAATAGGAGGCTTTTGTGAGGCCCTAGGCTTTTGCAAAAAGCTCCCGGGAGCTTGGATAT  
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PstI (3441)

GCCCCGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCTTGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGA  
 PvuII (3496)

CGGGAGTTCTTGGCAGCTGTGCTCGACCTGTCTACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGCGAGGATC  
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 ATAAAGCAATGCTATCACAATTTTCACTAATTAAGCATTTTTCATGCAATCTAGTGTGGTTTGTCCAACTCATATGATATC

BamHI (4352)

TTATCATGTCTGATCCCGTCGACCTCGAGGCTTGGGTAATCATGGTCTATAGCTGTCTTCTGTGTGAATTTGTATCCGCTCACA  
 ATTCCACACATATACGAGCCCGAGCTTAAGTGTAAAGCTGGGCTGCCATATGAGTGAGCTAATCTCATTAATTCGCTTGGCG

PvuII (4561)

TCACTGCCCGCTTTCAGTGGGAAACCTGTCTGCGCACTGCTTAATGAATCGGCCAACGCGGGGAGAGGGCGTTTGGGTATT  
 GGGCGCTCTTCCGCTTCTCTGCTCACTGACTCGCTGCGCTCGGTGTTGGCTGGGCGAGCGGTATCAGCTCATCTCAAGGGCGTA  
 ATACGGTTATCCACAGAACTCAGGGGATACGCGAGAAAGACCTGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAGGGCC  
 GCGTTGCTGGCGTTTTCCTAAGGCTCCGCCCCCTGAGCGAGCTACACAAAATGACGCTCAAGTCAAGGTTGGCGAAACCGGACA  
 GACTATAAAGTAACCGGCGTTCCCCCTGGAAGCTCCCTGCTGGCTCTCTGTTTCGGAACCTGCGCTTACCGGATACCTGTCC  
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 GGCTGTGTGACGAACCCCCGTTCAAGCCCAAGCTGGGCTTATCGGTAATCTGCTTGAAGTGGTGGGCT  
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 TTCACCTAGATCCTTTAAATTAAGTGAAGTTTAAATCAATCAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGC  
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 CGGGAGGGCTTCCATCTGCCCCAGTGTGCAATGATAACCGAGACCCAGCTCAACGGCTCCAGATTATCAGCAATAAACCAG  
 CCAGCCGGAGGGCCGAGCGCAGAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAAATGTTGCCCGGAGGCTAGAGTA  
 AGTAGTTCCGCTGTTAATAGTTTGGCAACGTTGTTGCCATGCTACAGGCATGTTGGTGTCAAGCTCGTCTGTTGGTATGGCTTCA

PvuII (!)

TTACGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTAGCTCTCTCCGCTCTCCGATC  
 GTTGTACAGAGTAAGTTGGCGCGAGTGTATCACTCATGGTATAGGCGACCTGCTAATTTCTCTACTGTCTATGCCATCCGTAAGA

ScaI (6110)

TGCTTTTCTGTGACTGGTGAATCTCAACCAAGTCAATCTGAGATAGTGTATGCGCGGACCGAGTTGCTCTTCCCGCGCTCAATA

XmnI (6227)

CGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTCATATGGAAACGTTCTTCCGGGCGGAAACTCTCAAGGATCTTA  
 CCGCTGTGAGATCCAGTTCCGATGTAACCCACTCGTGCACCACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGGGTGA  
 GCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACAAGGAAATGTTGAATACTCATACTCTTCTCTTTTCAATAT  
 TATTGAAGCATTTATCAGGCTTATTGTCTCATGAGCGGATACATATTTGAATGATTTAGAAAAATAAACAATAAGGGGTTCCCGCG  
 ACAATTCCTCCGAAAGTGCCACTGACGTC

**CLAIMS**

1. A molecular chimaera for use in therapy with a prodrug, the chimera comprising a transcriptional regulatory DNA sequence capable of being activated in a targetted mammalian cell and a DNA coding sequence operatively linked to the transcriptional regulatory DNA sequence and encoding a  $\beta$ -lactamase enzyme such that on expression of said coding sequence in the targetted cell, the  $\beta$ -lactamase enzyme is capable of catalysing conversion of the prodrug into an agent toxic to the targetted cell.
2. A molecular chimaera according to claim 1 wherein the transcriptional regulatory DNA sequence is tissue- or cancer-specific.
3. A molecular chimera according to claim 1 or 2 wherein the transcriptional regulatory DNA sequence comprises a promoter.
4. A molecular chimera according to claim 3 wherein the transcriptional regulatory DNA sequence also comprises an enhancer.
5. A molecular chimaera according to any of claims 1 to 4 wherein the transcriptional regulatory DNA sequence is selected from the transcriptional regulatory DNA sequences of the genes for albumin, alphafetoprotein, carcinoembryonic antigen, tyrosine hydroxylase, choline acetyl transferase, neuron specific enolase, glial fibrillary acid protein, insulin, gamma-glutamyltranspeptidase, dopa decarboxylase, HER2/neu, and N-myc.
6. A molecular chimera according to any of claims 1 to 5 wherein the targetted mammalian cell is a small cell lung carcinoma, non-small cell lung carcinoma, retinoblastoma, pheochromocytoma, medullary thyroid carcinoma, insulinoma, pituitary tumour, hepatoma, teratocarcinoma, gastrointestinal tumour cell or a testicular non-seminomatous carcinoma.
7. A molecular chimaera according to any of claims 1 to 5 wherein the targetted mammalian cell is a virally infected cell.

8. A molecular chimera according to claim 7 wherein the virally infected cell is infected with HIV.
- 5 9. A molecular chimera according to any of claims 1 to 8 which provides intracellular expression of the  $\beta$ -lactamase enzyme in the targetted cell.
- 10 10. A molecular chimera according to claim 9 wherein the sequence providing for intracellular expression of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 5.
- 11 11. A molecular chimera according to claim 10 wherein the sequence providing for intracellular expression of the  $\beta$ -lactamase enzyme has the sequence of SEQ ID NO 5.
- 15 12. A molecular chimera according to any of claims 1 to 8 which provides for secretion of the  $\beta$ -lactamase enzyme such that the enzyme is capable of extracellular conversion of the prodrug into an agent toxic to the targetted cell.
- 20 13. A molecular chimera according to claim 12 wherein the sequence providing for secretion of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 3.
- 25 14. A molecular chimera according to claim 13 wherein the sequence providing for secretion of the  $\beta$ -lactamase enzyme has the sequence of SEQ ID NO 3.
15. A molecular chimera according to claim 12 which provides for membrane bound expression of the  $\beta$ -lactamase enzyme such that the enzyme is capable of extracellular conversion of the prodrug into an agent toxic to the targetted cell.
- 30 16. A molecular chimera according to claim 15 wherein the sequence providing for membrane bound expression of the  $\beta$ -lactamase enzyme is at least 80% homologous to that of SEQ ID NO 9.

17. A molecular chimera according to claim 16 wherein the sequence providing for membrane bound expression of the  $\beta$ -lactamase enzyme has the sequence of SEQ ID NO 9.
- 5 18. A molecular chimera according to any of claims 1 to 17 for use with a prodrug which is a conjugate of a cephalosporin and an agent toxic to the targetted cell which is capable of being converted to the agent toxic to the targetted cell by the  $\beta$ -lactamase enzyme.
- 10 19. A molecular chimera according to claim 18 wherein the agent toxic to the targetted cell is methotrexate or 5-fluorouracil.
20. A vector containing a chimera as claimed in any of claims 1 to 19.
- 15 21. A packaging cell line containing a vector as claimed in claim 20.
22. An infective virion generated from a packaging cell line as claimed in claim 21.
- 20 23. An infective virion according to claim 22 which is a retrovirus, an adenovirus or an adeno-associated virus.
24. A packaging cell line capable of producing an infective virion as claimed in claim 23.
- 25 25. Use of an infective virion according to claims 22 or 23 for the manufacture of a medicament for use in therapy wherein the therapy comprises the selective infection of a targetted mammalian cell using retroviral infection, adenoviral infection or adeno-associated viral infection, physically localised delivery or engineered viral coat proteins.
- 30 26. A pharmaceutical formulation comprising a molecular chimera as claimed in any of claims 1 to 19, a vector as claimed in claim 20, a packaging cell line as claimed in claim 24 or an infective virion as claimed in claim 22 or 23.

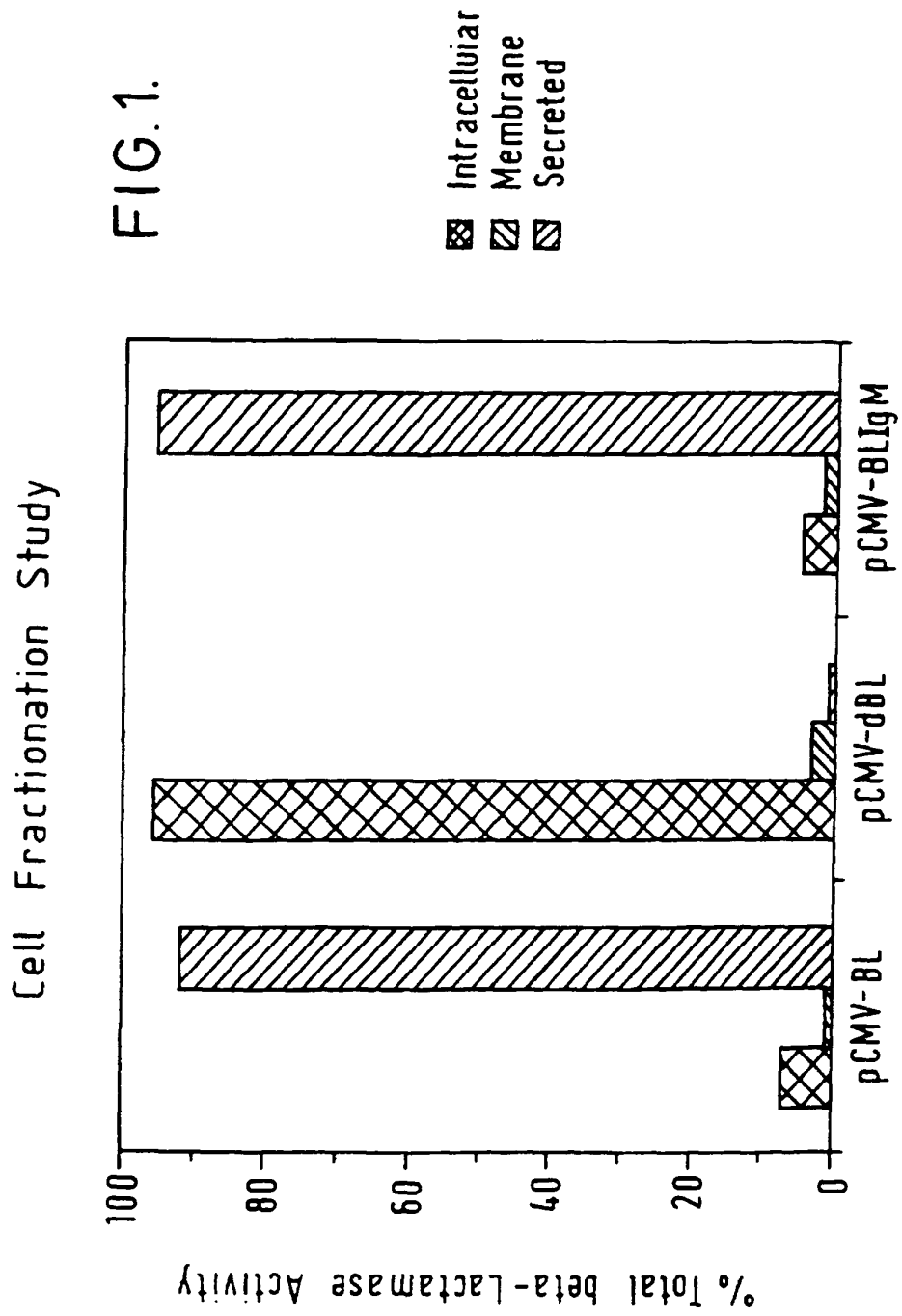
27. Use of a molecular chimaera according to any of claims 1 to 19 for the manufacture of a medicament for use in therapy wherein the therapy comprises administering said molecular chimaera by a method selected from calcium phosphate transfection, electroporation, microinjection, liposomal transfer, ballistic barrage, retroviral infection and adeno or adeno-associated virus infection.

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28. A method of treating cancer, a viral infection or an inflammatory condition which comprises administering to a mammal an effective amount of a chimaera, as claimed in any one of claims 1 to 19, a vector as claimed in claim 20, a packaging cell line as claimed in claim 24 or an infective virion as claimed in claim 22 or 23.

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1 / 1





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/85, 15/86, 9/86, 5/10, A61K 31/70, 47/48, 48/00</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 97/19180</b> <b>(43) International Publication Date:</b> 29 May 1997 (29.05.97)
<b>(21) International Application Number:</b> PCT/GB96/02845 <b>(22) International Filing Date:</b> 19 November 1996 (19.11.96) <b>(30) Priority Data:</b> 9523703.8      20 November 1995 (20.11.95)      GB <b>(71) Applicant (for all designated States except US):</b> GLAXO GROUP LIMITED [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> DEV, Inderjit [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). MOORE, John, Tomlin [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). OHMSTEDE, Carol-Ann, Dinsmore [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). <b>(74) Agent:</b> REES, Marion; Glaxo Wellcome plc, Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> <b>(88) Date of publication of the international search report:</b> 28 August 1997 (28.08.97)
<b>(54) Title:</b> VECTOR CONSISTING OF A TRANSCRIPTIONAL REGULATORY DNA SEQUENCE LINKED TO A DNA SEQUENCE ENCODING BETA-LACTAMASE FOR ENZYME PRODRUG THERAPY <b>(57) Abstract</b> <p>The invention relates to a molecular chimaera for use in therapy with a prodrug, the chimaera comprising a transcriptional regulatory DNA sequence capable of being activated in a targetted mammalian cell and a DNA coding sequence operatively linked to the transcriptional regulatory DNA sequence and encoding a <math>\beta</math>-lactamase enzyme such that on expression of said coding sequence in the targetted cell, the <math>\beta</math>-lactamase enzyme is capable of catalysing conversion of the prodrug into an agent toxic to the targetted cell. The enzyme <math>\beta</math>-lactamase has particular advantages when used in GDEPT or VDEPT in terms of the range of prodrugs that can be produced which are capable of being converted to the active species by the enzyme.</p>		

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# INTERNATIONAL SEARCH REPORT

In national Application No  
PCT/GB 96/02845

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/85 C12N15/86 C12N9/86 C12N5/10 A61K31/70  
A61K47/48 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)  
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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Y	---	1-28
Y	EP 0 415 731 A (THE WELLCOME FOUNDATION LIMITED) 6 March 1991 cited in the application see pages 3-7 ---	1-28
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